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The Effects of Smoking on Glutathione Levels in Bronchoalveolar Lavage Fluid

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Abstract: Cigarette smoke contains high concentrations of oxidants and reactive oxygen species. The aim of this study was to evaluate the effect of long-term cigarette smoking on glutathione (GSH) levels in bronchoalveolar lavage (BAL) fluid. Nineteen current cigarette smokers and seven healthy nonsmoking volunteers were included in the study. BAL was performed by infusion of five sequential 20 ml aliquots and each aliquot was immediately aspirated. The levels of GSH were determined in BAL fluids. The epithelial lining fluid volume was calculated by the ratio of urea concentration in BAL fluid to that in serum. Lower GSH levels were observed in the BAL fluid of current smokers than in that of nonsmokers (8.28 ± 0.78, 12.74 ± 1.09 μM respectively, $p<0.01$) GSH levels in epithelial lining fluid from smokers were also significantly lower than those from nonsmokers ($p<0.001$). Our data suggest that chronic smoke exposure causes decreased GSH levels in the BAL fluid of current smokers, possibly due to the excess generation of oxidants and/or impairment of the synthesis of antioxidants at different cellular levels. The oxidant and antioxidant imbalance observed in smokers may be a factor in the pathogenesis of oxidant-mediated damage to respiratory airways and lungs.

Key Words: Bronchoalveolar lavage, Chronic obstructive pulmonary disease, Glutathione, Smoking.

Introduction

Cigarette smoking is a predominant etiologic factor for chronic obstructive pulmonary disease (COPD) (1). Tobacco smoke contains high concentrations of oxidants and reactive oxygen species, present in both the gas phase and the tar phase. It has been estimated that each puff of smoke contains 10^{16} oxidants (2-5). Furthermore, alveolar macrophages and neutrophils in the lower respiratory tract of smokers have been found increase by 2-4 times and 10 times respectively (6). These inflammatory cells release additional oxidants capable of causing cell injury. Tobacco smoke, therefore, leads to an increased load of oxidants on the respiratory tract, both directly by the inhalation of oxidants in the smoke and indirectly by the activation of inflammatory cells (2, 5).

The lung contains antioxidant defense systems against oxidants (2, 5, 7-9). Glutathione (GSH), a sulphydryl-containing tripeptide is abundant in the epithelial lining fluid (ELF), which covers the respiratory epithelial cells. It protects the oxidation of the sulphydryl group of some proteins and peroxidation of membrane polyunsaturated fatty acids (2, 5, 7-10).

There are few studies on the GSH levels in the ELF of current cigarette smokers. Therefore, the aim of this study was to determine the effects of long-term smoking on the local antioxidant defense system in the bronchoalveolar lavage (BAL) fluid of current smokers, with a focus on GSH levels.

Materials and Methods

Nineteen current smokers with evidence of COPD (14 male, 5 female) (mean age ± SEM, 50.32 ± 2.71 yr.) and seven healthy nonsmoking volunteers (1 male, 6 female) (mean age ± SEM, 44.14 ± 2.84 yr.) were recruited from the outpatient clinic of the Department of Chest Diseass in the Hospital of Osmangazi University. Informed consent was obtained.

Clinical findings and results of physical examinations were in accordance with the criteria of American Thoracic Society (ATS) for COPD in current smokers (11). The

results of electrocardiogram, urinalysis and hematological and blood chemistry screening tests were normal. None of the subjects had respiratory tract infection of acute exacerbation of COPD for 1 month prior to the study. Of the 19 current smokers, 13 Chest x-rays revealed pathological changes due to emphysema in 13 of the 19 current smokers (Small, pendulous heart; low, flat diaphragms; areas of increased radiolucency). Fourteen of the 19 subjects consumed more than 20 cigarettes per day and had an average smoking history of 33.89 ± 3.98 pack-years.

All subjects underwent both routine pulmonary function testing (Fudac 50 spirometry, Analyzer ST 90 spirometry) and arterial blood gas analysis (Eschweiler System 2000) prior to BAL procedure. Venous blood samples were obtained from the subjects for the determination of urea levels.

BAL was performed with standard techniques. Subjects were premedicated with atropine and diazepam. Bronchoscopy was performed through the oral route (Olympus BF Type 2T 10) under local anesthesia with 10% xylocaine and 0.1% pantocaine. The bronchoscope was wedged in medial or lateral subsegment of the right middle lobe and 100 ml sterile saline was instilled in 5 aliquots of 20 ml each. After instillation, each aliquot was immediately aspirated. The fluid recovered was collected in polyethylene tubes kept on ice. The recovered fluid was quantitated and filtered through two single layers of sterile gauze and centrifuged at 500 rpm for 10 min at 4°C in order to separate the cellular constituents.

Total GSH was measured according to the method of Beutler (12). The reaction of GSH with 5-5'-dithiobis (2-nitrobenzoic acid) was monitored at 412 nm.

Urea concentrations in BAL fluid and in sera were determined by BM-Hitachi 911 automated analyzer with the original kits of Boehringer-Mannheim based on the urease method. The volume of ELF was calculated by the following formula (13):

ELF = BAL urea / sera urea X recovered BAL volume

The results are expressed as a mean (SEM). Statistical analysis was carried out by Student's t test and $p < 0.05$ was considered statistically significant. Correlations were analyzed by the Pearson test.

Results

The group of current smokers was slightly older than the group of normal subjects, but not significantly so. Arterial blood gas analysis revealed that there was no statistically significant difference in the PaO_2, $PaCO_2$ and O_2 saturation between the two groups.

The mean values of all spirometry parameters are listed in Table 1. The pulmonary function test parameters of the current smokers were, of course, significantly lower than those of nonsmokers, except FEV_1 / FVC (%).

Current smokers had a lower proportion of instilled BAL fluid recovered (48.16 ± 2.86 ml) than did nonsmokers (53.00 ± 3.94 ml). However, this difference was not significant statistically. Current smokers had ELF levels that were even greater (1.48 ± 0.20 ml) than those observed in the nonsmokers (0.95 ± 0.21 ml) (Table 2).

The concentration of GSH was significantly lower in the BAL fluid of current smokers than in that of nonsmokers (8.28 ± 0.78 μM, 12.74 ± 1.09 μM respectively, $p < 0.01$).

The concentration of GSH in ELF is shown in Table 2. The composition of BAL fluid differed significantly between current smokers and controls, regardless of whether it was expressed as a concentration in BAL fluid or compared to ELF levels.

There was a significant inverse correlation between the number of pack-years and the pulmonary function test parameters, except FEV_1 / FVC (%) (Table 3). There was also a significant negative correlation between the ELF volume and the number of pack-years. The volume of BAL fluid correlated positively with FEV_1, FEV_1 / FVC (%), PEF, DLCO and kCO, as shown in Table 3. The concentration of GSH in BAL fluid correlated positively with the FEV_1, VC and FVC. In contrast, the concentration of GSH in BAL fluid was inversely correlated with the number of pack-years (Figure 1).

Table 1. The results of pulmonary function tests (Predictive value %) (Mean ± SEM).

	Controls	Smokers
VC	97.28 ± 4.48	77.32 ± 3.66*
FVC	95.29 ± 6.28	73.58 ± 3.90*
FEV_1	97.57 ± 6.45	70.95 ± 4.89*
FEV_1 / FVC (%)	103.29 ± 5.3	88.47 ± 4.15
PEF	89.43 ± 7.35	61.47 ± 4.29*
DLCO	100.71 ± 2.81	80.88 ± 3.65*
kCO	104.43 ± 5.57	85.14 ± 4.41‡

* As compared to controls $p < 0.01$

‡ As compared to controls $p < 0.05$

Table 2. Concentrations of antioxidants in BAL fluid (Mean ± SEM).

	Controls	Smokers
Recovered Volume (ml)	53.00 ± 3.94	48.16 ± 2.86
ELF Volume (ml)	0.95 ± 0.21	1.48 ± 0.20
GSH (μM)	12.74 ± 1.09	8.28 ± 0.78*
ELF GSH (μM)	902.43 ± 174.85	307.84 ± 32.22†

† As compared to controls $p < 0.001$

* As compared to controls $p < 0.01$

Discussion

In this study, we found that current smokers have decreased antioxidant defenses, as reflected by lower levels of GSH in BAL fluid than those of normal non-smoking subjects.

Oxidants in cigarette smoke play an important role in the injury of the lower respiratory system cells. The reduction of intracellular antioxidants of lung cells exposed to oxidants in cigarette smoke have previously been shown in an in vitro study (5). Moreover, smoking causes a chronic inflammation in the lower respiratory tract epithelium (14, 15). Cigarette smoke can activate alveolar macrophages and the alternate pathway of the complement, in addition to the induction of polymorphonuclear leukocyte (PMN) chemotaxis and activation (4, 16). The activated alveolar macrophages and PMNs are recruited in the lower respiratory system of cigarette smokers, and oxidants are also produced by these activated cells (14, 15, 17).

Table 3. Correlation coefficients (r) between different parameters.

	Pack-years	BAL Volume	GSH
FEV1	–0.543‡	0.693*	0.505‡
FEV1 / FVC (%)	—	0.688*	—
PEF	–0.467‡	0.518‡	—
VC	–0.474‡	—	0.584*
FVC	–0.471‡	—	0.585*
DLCO	–0.549‡	0.547‡	—
kCO	–0.604*	0.519‡	—
ELF Volume	–0.589*	—	—
Pack–years	—	—	–0.537‡

* As compared to controls $p < 0.01$

‡ As compared to controls $p < 0.05$

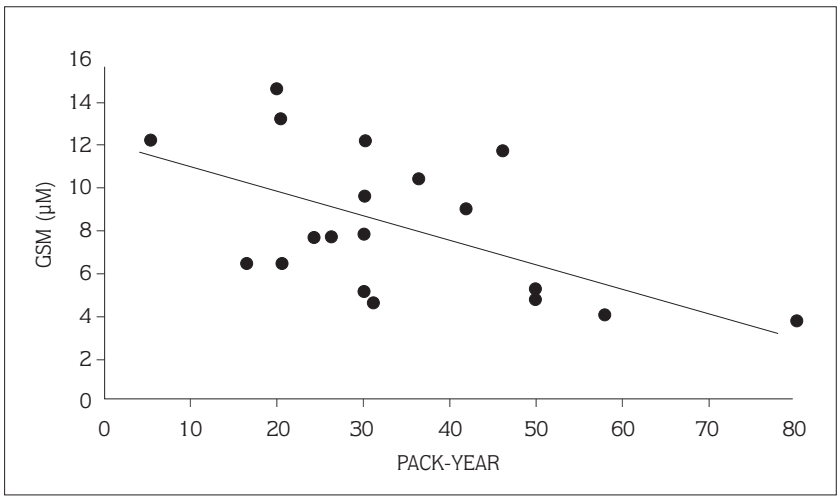

Figure 1. Negative correlation between GSH concentration and cigarette consumption (pack-years).

GSH is the most abundant tripeptide thiol in cells and protects the cellular components from the effects of hydrogen peroxide and other hydroperoxides by providing reducing equivalents. Recently, high levels of GSH were shown in ELF (10). Cantin et al. have shown higher levels of GSH in ELF in smokers with a mean cigarette consumption of 22 pack-years and a mean age 31 years than in controls (10). Furthermore, smokers n that study had no abnormal findings in chest radiographic and spirographic examinations. The discrepancy between the GSH results of Cantin et al. and those of our study is possibly due to the higher mean age and cigarette consumption of smokers in our study (50 years and 33 pack-years respectively). It is generally considered that airway obstruction and bronchus hyperreactivity are the most prominent findings of long-term exposure to cigarette smoke (18). In our study, the smokers had pathological findings in their chest x-rays and spirometric examinations, which support the airway obstruction hypothesis. Moreover, the cigarette consumption period and pack-years are the most important causitive factors in pathological changes in airways and lungs (19-22). The significant negative correlation between GSH levels and pack-years and the significant positive correlation between GSH levels and FEV_1, FVC and VC in our study is consistent with this knowledge.

Linden et al. put the smokers into obstructive and non-obstructive groups with a mean age 55 years and a mean cigarette consumption of 35 pack-years, and found slightly lower levels of GSH in the ELF of obstructive smokers than in non-obstructive smokers (14). The mean GSH level of current smokers in our study is consistent with the results of Linden et al., especially in that our study group had obstructive findings in their chest x-rays and spirometric examinations. Since Linden and coworkers had no non-smoker control group in the mentioned study, we were unable to compare our non-smoker results with theirs.

We hypothesize that smoking causes a compensatory increase of ELF GSH levels until pathological changes of lung and airways arise. However, long-term exposure to cigarette smoke causes a reduction of GSH levels in ELF accompanied by pathological changes of respiratory tract. The decreased levels of GSH in the BAL fluid of smokers might be due to the consumption of GSH during the detoxification of oxidants in cigarette smoke. It has been shown that an increased burden of oxidants consumes the antioxidants during chemical conjugation (5).

McCusker and Hoidal showed increased antioxidant enzyme activities in order to compensate the oxidant stress in alveolar macrophages of smokers (23). However, this increase was reversible, so the enzyme activities returned to control levels 4 weeks after smoke exposure stopped. These observations observations support our hypothesis that smoking causes a compensatory increase of antioxidant defenses in the early period, but that long-term cigarette smoking shifts the oxidant-antioxidant balance toward decreased antioxidant defenses in current smokers, particularly those with pathological changes in their respiratory tracts.

In this regard, the antioxidant systems in the BAL fluid of young smokers can protect the lungs against oxidant stress, whereas continued smoking in the elderly may reduce the compensatory elevation of antioxidants that cause cellular injury.

Our data suggest that chronic smoke exposure causes decreased GSH levels in BAL fluid from smokers, possibly due to the excess generation of oxidants and/or the impairment of the synthesis of antioxidant enzymes or proteins at different cellular levels. The oxidant-antioxidant imbalance observed in smokers may be a factor in the pathogenesis of oxidant-mediated damage to respiratory airways and alveolar structures. Further studies including young and elderly smokers with obstructive changes in their lower respiratory tracts are required to clarify the mechanisms.

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